

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
4 July 2002 (04.07.2002)

PCT

(10) International Publication Number  
**WO 02/052271 A3**

(51) International Patent Classification<sup>7</sup>: **G01N 33/68**

(21) International Application Number: PCT/EP01/15228

(22) International Filing Date:  
21 December 2001 (21.12.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:  
60/257,559 22 December 2000 (22.12.2000) US  
60/332,965 19 November 2001 (19.11.2001) US

(71) Applicant (for all designated States except AT, US): **NOVARTIS AG** [CH/CH]; Lichtstrasse 35, CH-4056 Basel (CH).

(71) Applicant (for AT only): **NOVARTIS-ERFINDUNGEN VERWALTUNGSGESELLSCHAFT MBH** [AT/AT]; Brunner Strasse 59, AT-1230 Vienna (AT).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **WANG, Yingqi,**

**Karen** [US/US]; 16 Heritage Drive, East Hanover, NJ 07936 (US). **MA, Zhixiang** [CN/US]; 100 Center Grove Road, Apt. 2-12, Randolph, NJ 07869 (US). **QUINN, Douglas, Frederick** [US/US]; 14 Springview Drive, Morristown, NJ 07960 (US). **FU, Emil, W.** [US/US]; 14 Daniel Drive, East Hanover, NJ 07936 (US).

(74) Agent: **BECKER, Konrad**; Novartis AG, Corporate Intellectual Property, Patent & Trademark Department, CH-4002 Basel (CH).

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LT, LU, LV, MA, MD, MK, MN, MX, NO, NZ, OM, PH, PL, PT, RO, RU, SE, SG, SI, SK, TJ, TM, TN, TR, TT, UA, US, UZ, VN, YU, ZA, ZW.

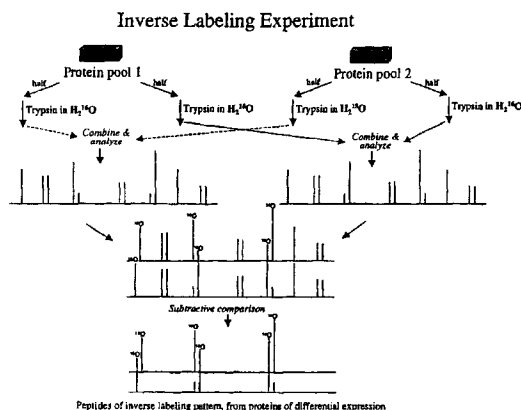
(84) Designated States (*regional*): Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR).

Published:

— with international search report

[Continued on next page]

(54) Title: INVERSE LABELING METHOD FOR THE RAPID IDENTIFICATION OF MARKER/TARGET PROTEINS



(57) **Abstract:** A novel procedure for performing protein labeling for comparative proteomics termed inverse labeling is provided for the rapid identification of marker or target proteins. With this method, to evaluate protein expression of a disease or a drug treated sample in comparison with a control sample, two converse collaborative labeling experiments are performed in parallel. In one experiment the perturbed sample (by disease or by drug treatment) is isotopically heavy-labeled, whereas, the control is isotopically heavy-labeled in the second experiment. When mixed and analyzed with its unlabeled or isotope light counterpart for differential comparison, a characteristic inverse labeling pattern is observed between the two parallel analyses for proteins that are differentially expressed to an appreciable level. In particularly useful embodiments, protein labeling is achieved through proteolytic <sup>18</sup>O-incorporation into peptides as a result of proteolysis performed in <sup>18</sup>O-water, metabolic incorporation of <sup>15</sup>N (or <sup>13</sup>C and <sup>2</sup>H) into proteins, and chemically tagging proteins with an isotope-coded tag reagent such as an isotope-coded affinity tag reagent.



**(88) Date of publication of the international search report:**  
31 October 2002

*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

## INTERNATIONAL SEARCH REPORT

International Application No.

PCT/EP 01/15228

A. CLASSIFICATION OF SUBJECT MATTER  
IPC 7 G01N33/68

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

EPO-Internal, PAJ, WPI Data, MEDLINE, BIOSIS, EMBASE, SCISEARCH, CHEM ABS Data

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages   | Relevant to claim No. |
|------------|--|-----------------------|
| A          | <p>ROSE, K. ET AL: "A new mass-spectrometric C-terminal sequencing technique finds a similarity between gamma-interferon and alfa2-interferon and identifies a proteolytically clipped gamma-interferon that retains full antiviral activity" THE BIOCHEMICAL JOURNAL, vol. 215, no. 2, 1 November 1983 (1983-11-01), pages 273-277, XP001079683 cited in the application the whole document</p> <p style="text-align: center;">---<br/>-/--</p> | 1-49                  |



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

## \* Special categories of cited documents :

\*A\* document defining the general state of the art which is not considered to be of particular relevance

\*E\* earlier document but published on or after the International filing date

\*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

\*O\* document referring to an oral disclosure, use, exhibition or other means

\*P\* document published prior to the international filing date but later than the priority date claimed

\*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

\*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

\*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

\*&amp;\* document member of the same patent family

Date of the actual completion of the international search

25 July 2002

Date of mailing of the International search report

13/08/2002

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax: (+31-70) 340-3016

Authorized officer

Moreno de Vega, C

## INTERNATIONAL SEARCH REPORT

International Application No.

PCT/EP 01/15228

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages  | Relevant to claim No. |
|------------|---|-----------------------|
| A          | ODA, Y. ET AL: "Accurate quantitation of protein expression and site-specific phosphorylation"<br>PROC. NATL. ACAD. SCI.,<br>no. 96, 1999, pages 6591-6596, XP001080471<br>cited in the application<br>the whole document   | 1-49                  |
| A          | JI, J. ET AL: "Strategy for qualitative and quantitative analysis in proteomics based on signature peptides"<br>JOURNAL OF CHROMATOGRAPHY,<br>no. 745, 2000, pages 197-210, XP004215299<br>the whole document   | 1-49                  |
| A          | CHEN, X. ET AL: "Site-specific mass tagging with stable isotopes in proteins for accurate and efficient protein identification"<br>ANAL. CHEM.,<br>vol. 72, no. 6,<br>15 March 2000 (2000-03-15), pages<br>1134-1143, XP002207456<br>cited in the application<br>the whole document | 1-49                  |
| P,A        | WO 01 94935 A (MDS PROTEOMICS)<br>8 June 2001 (2001-06-08)<br>claims 1-61   | 1-49                  |
| P,X        | WANG, Y.K. ET AL: "Inverse 18 O labeling mass spectrometry for the rapid identification of marker/target proteins"<br>ANALYTICAL CHEMISTRY,<br>vol. 73, no. 15,<br>1 August 2001 (2001-08-01), pages<br>3742-3750, XP001061446<br>USA<br>the whole document                         | 1-49                  |

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 01/15228

| Patent document<br>cited in search report | Publication<br>date | Patent family<br>member(s) | Publication<br>date |
|---|---------------------|----------------------------|---------------------|
| WO 0194935 A                              | 13-12-2001          | AU 7094101 A               | 17-12-2001          |
|   |                     | WO 0194935 A2              | 13-12-2001          |
|   |                     | US 2002076817 A1           | 20-06-2002          |
| <hr/>                                     |                     |                            |                     |